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Title: Loss of dopaminergic nigrostriatal neurons accounts for the motivational and affective deficits in Parkinson's disease.

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Abstract

Parkinson's Disease (PD) involves the degeneration of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc) that is thought to cause the classical motor symptoms of this disease. However, motivational and affective impairments are also often observed in PD patients. These are usually attributed to a psychological reaction to the general motor impairment and to a loss of some of the neurons within the ventral tegmental area (VTA). We induced selective lesions of the VTA and SNc DA neurons that did not provoke motor deficits, and showed that bilateral dopamine loss within the SNc, but not within the VTA, induces motivational deficits and affective impairments that mimicked the symptoms of PD patients. Thus, motivational and affective deficits are a core impairment of PD, as they stem from the loss of the major group of neurons that degenerates in this disease (DA SNc neurons) and are independent of motor deficits.

Main text:

Parkinson's disease (PD) is mainly characterized by a progressive degeneration of midbrain dopaminergic (DA) neurons along a caudorostral and lateromedial gradient, with a marked loss of neurons in the substantia nigra pars compacta (SNc)¹, projecting to the dorsal striatum along the nigrostriatal pathway², and a more modest loss in the ventral tegmental area (VTA)¹, projecting to limbic and cortical areas along the mesolimbic and mesocortical pathways², respectively.

In addition to the classical motor symptoms, several neuropsychiatric symptoms, such as depression, anxiety and motivational deficits (apathy), are frequently observed in PD patients^{3, 4}. The underlying pathological mechanisms have not yet been elucidated, but these motivational and affective impairments are generally attributed to the patient's psychological reaction to the profound motor deficit and to the associated loss of dopaminergic neurons in the VTA^{4, 5}. This pathophysiological concept stems from a dichotomous vision of the functional role of mesencephalic DA neurons, in which motor function is attributed to the nigrostriatal system, originating from the SNc, and motivational and affective functions are attributed to the mesocorticolimbic system, originating from the VTA^{2, 6, 7}.

Most of the data that have been used to ground this dichotomous vision of VTA-SNc neurons implication in PD symptoms have been generated using lesions of these neurons, involving various degrees of destruction of bordering zone of the VTA and SNc. Therefore, large SNc lesions inducing profound motor deficits also involve the lateral part of the VTA. Conversely, the VTA lesions used to attribute motivational properties to this structure always involve destruction of the medial part of the SNc^{6, 8}. Consequently, the origins of the motivational symptoms in PD remain unresolved, not least because disentangling the potential motivational and mood-related deficit alterations from motor impairments remains a challenging issue in animal models of PD⁹.

We have developed a lesional model, based on the stereotaxic injection of the catecholaminergic neurotoxin 6-hydroxydopamine (6-OHDA) into precise areas of the rat brain, in which degenerations of the DA mesocorticolimbic and nigrostriatal systems can be clearly separated

and in which the motor skills of the animals are preserved. We evaluated several aspects of behavior and found that bilateral dopamine loss within the SNc, but not within the VTA, caused motivational deficits and affective impairments resembling some of the neuropsychiatric symptoms observed in PD patients.

Results

Bilateral partial lesions of the mVTA or SNc result in distinct, non overlapping, complementary patterns of DA denervation

mVTA and SNc lesions produce two distinct, non overlapping, complementary, patterns of bilateral DA denervation throughout the striatum, as revealed by decreases in tyrosine hydroxylase (TH)-immunoreactivity (IR) and striatal DA contents (Fig. 1 and Fig. S1). The mVTA lesions preferentially affected the ventral part of the striatum, resulting in a 40 to 60% decrease in TH-IR density within the nucleus accumbens. The extent of the mVTA lesions therefore mimicked the loss of dopaminergic innervation observed in the ventral head of the caudate nucleus in PD patients¹⁰. The SNc lesion was associated with a similar decrease in TH-IR density, along the rostrocaudal extent of the dorsal striatum, predominantly in its lateral portion (~70%).

Bilateral partial DA lesions of the mVTA or SNc do not induce locomotor deficits

As expected with such partial striatal DA denervation¹¹, neither SNc nor mVTA lesions impaired sensorimotor coordination on an accelerating rotarod or spontaneous locomotor activity (Fig. 2A, B). Furthermore, despite a slight alteration of stepping adjustment in SNc DA-lesioned rats (Fig. 2C), no impairment was observed with regards to fine-motor velocity or ambulatory coordination in an automated laboratory gait analysis system (Fig. 2D).

Bilateral partial DA lesions of the SNc, but not of the mVTA, decrease general behavioral activity

The lack of a major motor deficit after partial lesioning of the SNc allowed us to study specifically the role of the DA nigrostriatal system in motivational processes, in the absence of the usual potential bias related to locomotor alterations. Apathy is one of the major non motor symptoms of PD^{3, 4}. Apathy is defined as a lack of motivation, or a reduction in “goal-directed behaviors”¹², with a global deficit in self-initiation and maintenance of voluntary and purposeful behavior, resulting in low levels of activity and a

loss of interest in sources of reinforcement¹³⁻¹⁵. Interestingly, the mVTA lesions had no significant effect on general consummatory responses, measured as water and food intake over a 24-hour period, whereas SNc lesions decreased these behaviors (Fig. 3A and B) and were associated with a slower weight gain (Fig. 3C), sometimes associated with a transient starvation state after surgery (see *online Methods*). Like apathetic patients^{12, 13}, rats with DA lesions of the SNc, but not of the mVTA, displayed a decrease in social interaction (Fig. 3D) that could not be attributed to an olfactory deficit, since neither attraction to an appetitive odor (coconut, Fig. 3E) nor avoidance from an aversive (acetic acid, Fig. 3F) odor was altered by the DA lesions.

Bilateral partial DA lesions of the SNc, but not of the mVTA, specifically impair motivated behaviors

This aspect of behavior was investigated further, in various non operant and operant tasks, including place preference, instrumental responding and runway tasks for palatable food. The use of this approach made it possible to distinguish between the effects of the lesion on preparatory and consummatory components of motivated behaviors. Specifically, the acquisition of a runway task (progressive reduction in the latency to reach and to start to eat a palatable food at the end of a straight alley) was similar in all conditions, demonstrating an absence of learning deficits (Fig. 4A). However, SNc DA lesions decreased asymptotic performance, essentially due to a greater number of interruptions and route reversals in SNc-lesioned than in sham rats, resulting in a smaller number of direct runs to the goal (Fig. 4A). These observations are consistent with a weaker motivation to reach the goal¹⁶ or an approach/avoidance conflict¹⁷, that cannot be attributed to deficits in Pavlovian associative processes or in the reinforcing properties of the palatable food, as neither type of lesion affected performance in conditioning place preference (CPP) for the same food (Fig. 4B).

An effect of SNc lesion on preparatory behavior was further demonstrated by a dramatic impairment of instrumental responding for a sucrose solution in SNc lesioned rats as compared to both sham and mVTA lesioned rats (Fig. 4C and S3A-B), the latter even tending to outperform controls, both during acquisition and when the workload required to obtain the reward increased exponentially under a progressive ratio schedule of reinforcement, an index of motivation¹⁸ (Fig. S3C).

The reduced behavioral responses of SNc-lesioned rats cannot be attributed to an impairment in instrumental learning, as their capacities to discriminate between the active and inactive (control) lever throughout the task were preserved (Fig. S3B), or to a decrease in sensitivity to the motivational properties of sucrose, as demonstrated by their clear preference for the same sucrose solution in a two-bottle choice procedure (Fig. 4D and S4A). Similar results were obtained with saccharin, a non caloric sweetener (Fig. S4B), excluding an effect of potential metabolic confounding factors. In this test, lesioned animals were also able to differentiate between two saccharin concentrations and to shift their preference toward the concentration supplying the greatest reward (Fig. S4C). Thus, the poorer operant performances of the SNc lesioned animals do not result from a learning deficit or a change in reward or hedonic processing. Instead, they reflect a profound decrease in motivation to work to obtain the reward.

This deficit in the motivational preparatory responding also affects novelty-seeking, operationalized by the acquisition of instrumental conditioning reinforced only by contingent presentations of a “novel” cue-light¹⁹. As shown on Fig. 4E, this cue-light acted as a robust positive reinforcer in both sham groups and mVTA-lesioned rats, but not in SNc-lesioned animals. This result is of particular interest because neither mVTA nor SNc lesions impaired preference for a novel environment in a non instrumental novelty preference procedure (Fig. 4F), thereby suggesting that interest for novelty was unaffected. Again, a marked motivational deficit was observed specifically in animals with SNc DA lesions, when an instrumental preparatory action was required.

These between-subject differences specific to the SNc lesioned rats were further supported by dimensional analyses. Thus, whereas linear regression analyses yielded no significant correlations in the runway (data not shown), robust negative correlations were found between operant performances in the sucrose (Figure 4C) and cue-light self-administration procedure (Figure 4E) and the loss of TH immunoreactivity within the dorsal striatum for the SNc group. Interestingly, these correlations were not found within the NAc for the VTA group. These data therefore strengthen the implication of nigrostriatal DA in motivated behaviors.

Bilateral partial DA lesions of the SNc, but not of the mVTA, induce depressive and anxiety-related behaviors

This generalized lack of motivation seems to present face validity with regards to the pathophysiology of PD, which is frequently accompanied by mood disorders related to DA denervation, including depression and anxiety³⁻⁵. Consistent with observations in humans, SNc-lesioned rats also displayed a depressive-like behavior, as revealed by an increase in the time they spent immobile in the forced-swim test, whereas no such effect was observed in mVTA-lesioned rats (Fig. 5A and S5A). Similarly, anxiety-related behaviors, reflected by a reduced latency to enter in the dark side of a light/dark apparatus (Fig. 5B and S5B) and a decreased time spent in the open arms of an elevated plus-maze (Fig. 5C and S5C), were seen in SNc-, but not mVTA-lesioned animals. Therefore, only SNc DA lesions affected mood-related behaviors.

For these affective-related behaviors, a significant negative correlation was found between the latency to enter into the dark chamber in the light/dark avoidance test and loss of TH immunoreactivity within the dorsal striatum (Figure 5B) suggesting that the observed increase in anxiety is related to the degree of striatal dopamine depletion. However, no similar correlations were found in the elevated plus-maze and the forced-swim test (Figure 5A,C).

Reversion of the behavioral deficits resulting from nigrostriatal DA denervation by pharmacological DA agents

To further investigate the role of DA and validate our experimental approach, we tested whether subchronic administration of pharmacological DA agents classically used in PD could reverse some of the behavioral impairments induced by SNc lesions. The depressive- and anxiety-like behaviors displayed by SNc-lesioned rats were reversed by the D2/D3 agonist ropinirole and by L-dopa (Fig. 6A-B and S6A-B). Both pharmacological DA agents have been shown to have beneficial effects on apathetic symptoms and mood in PD^{3, 15}. These findings thereby provide an interesting predictive validity for this model, in terms of the causal implication of DA. In addition, ropinirole clearly improved motivated behaviors of SNc-lesioned rats, in an operant sucrose self-administration procedure (Fig.

6C and S6C-D). The selective serotonin reuptake inhibitor (SSRI) citalopram had no effect on motivated and depressive-like behaviors in lesioned animals (Fig. 6A and C, and S6), consistent with a selective role for DA, although the effect of this drug on anxiety-like behaviors (Fig. 6B) suggests a possible interaction with the serotonergic system.

Discussion

This study provides new insights into the pathophysiological mechanisms underlying the neuropsychiatric symptoms of PD. Indeed, our data clearly demonstrate that selective bilateral and partial lesions of the SNc, but not of the mVTA, induce a profound deficit in motivated behaviors and related affective changes. These behavioral deficits were selectively reversed by L-dopa and by the direct activation of the D2/D3 receptors with ropinirole, confirming the critical role played by DA.

Studying the non motor functions of the DA nigrostriatal system has remained a critical issue, due to the potential influence of motor deficits on the behavioral performances of the animals and the difficulty of targeting this pathway specifically. For example, large lesions of the DA nigrostriatal system and genetically-induced complete DA depletion lead to the so-called “lateral hypothalamic syndrome”, characterized by a dramatic aphagic and adipsic state^{6,20,21}, a phenotype in some ways similar to the decrease in consummatory responses we observed in SNc-lesioned animals. However, it was not clear whether these impairments resulted from a pure motivational deficit or from the strong akinesia induced by the lesion, and whether they were specific to the nigrostriatal system. Similarly, previous studies attempting to model cognitive dysfunctions and neuropsychiatric symptoms in PD with unilateral or bilateral 6-OHDA lesions in rats or MPTP-lesioned monkeys, have rarely been able to circumvent possible motor confounding factors and did not systematically aim to target the DA nigrostriatal system selectively^{9, 22, 23}. In the present study, with the use of partial and bilateral DA denervation, we were able to disentangle the non motor from the motor function, an approach that was further confirmed by the lack of specific relationship between individuals presenting no or mild motor deficits and their behavioral performances (data not shown). Thus, the lack of effect of the 6-OHDA lesions on several aspects of sensorimotor and ambulatory behavior suggests that motor alterations cannot account for the strong phenotype associated with partial DA denervation of the nigrostriatal system evidenced in this study.

These results therefore clearly demonstrate that selective bilateral lesions of the SNc, but not of the mVTA, induce a profound deficit in motivated and affective-related behaviors. While the lack of a robust correlation between affective-related deficits and dorsal striatum DA depletion suggests a potential role of extra-striatal regions receiving DA inputs from the SNc such as the amygdala or the

orbitofrontal cortex² in such behaviors, the clear-cut correlation between deficits in operant performances and the degree of TH loss in the dorsal striatum univocally emphasizes the predominant role of the DA nigrostriatal system in specific motivational processes. This result is particularly striking as, by contrast, a partial DA lesion of the mVTA, an area known to be involved in reinforcement and motivational processes^{6, 24-26}, did not modify any of the non motor behaviors evaluated here. Interestingly, sensitivity to reward and Pavlovian processes, which are dependent on the DA mesocorticolimbic system^{24, 26}, were unaffected by the lesion, which seems to alter the preparatory aspect of instrumental responses in a specific manner. Consistent with these findings, the SNc is located at the interface between the neurobiological systems underlying goal-directed and habitual control of behavior^{24, 27, 28}, where DA neurons encode crucial motivational and reward-related signals³⁰. The lesion generated is therefore likely to strongly interfere with the chain of processes that increase motivation and energize actions for a specific goal.

The behavioral phenotype induced by the SNc DA lesion is also reminiscent of apathy and related neuropsychiatric symptoms observed in PD patients^{4, 5}, suggesting that the DA nigrostriatal system plays a primary role in non motor deficits in PD. It has been suggested that apathy in PD may result from a corticostriatal dysfunction linked to the loss of nigrostriatal DA tone¹³. Furthermore, a recent study using an implicit incentive task¹⁴ reported an association of apathy in PD and non PD patients with changes in the motivational processes normally responsible for translating expected reward into effort and action, with no change in the perception of reward value. These findings are therefore strikingly similar to the motivational deficits observed in our experimental model and its underlying neurobiological substrate.

The adverse phenotype induced by the SNc lesion is reversed, at least partly, by pharmacological DA agents known to have positive effects on apathetic symptoms and mood in PD^{3, 15}. While both L-dopa and ropinirole completely reversed depressive- and anxiety-related behaviors, only ropinirole significantly improved motivated behaviors in lesioned animals. This may reflect the differences in the pharmacological properties of the two DA drugs, with L-dopa acting presynaptically on a denervated system and ropinirole having a stronger effect through the direct activation of

postsynaptic receptors, including, in particular, the D3 receptor, a potent regulator of mood and motivated behaviors²⁹. Indeed, it has been suggested that D2/D3 agonists such as ropinirole, may be more effective than other DA agents for the treatment of apathy, due to their high affinity for D2 and D3 receptors^{3, 15}. By contrast, the SSRI citalopram had no beneficial effect on motivated and depressive-like behaviors, consistent with several clinical observations suggesting that SSRIs are much less effective than DA agonists for reducing depressive symptoms in PD^{30, 31} and may even have a deleterious effect on apathy³². These data strengthen the validity of our experimental approach in regard to PD-related neuropsychiatric symptoms and the critical role of DA.

The effect of citalopram on anxiety-related behaviors indicates however a possible implication of the serotonergic system. Electrophysiological and neurochemical studies have reported a strong interaction between the dopaminergic and serotonergic systems, with a prominent modulation of midbrain DA neuronal activity by the raphe nuclei³³. Moreover, complex interactions between the serotonergic and dopaminergic system has been reported in PD³⁴ and related animal models³⁵. Such interactions may account for the complex influence of serotonin on the DA dysfunction highlighted by the present data.

In conclusion, this study highlights a critical role in motivation for the SNc that had been previously largely neglected and attributed to the DA mesoaccumbal pathway^{2, 27}. These data also demonstrate that motivational and affective deficits are a core impairment of PD, independent of the motor deficits and resulting from the loss of the major neuronal group known to degenerate in this disease (DA SNc neurons). This new insight into the pathophysiological mechanisms of mood and motivational dysfunctions in PD will facilitate the design of new treatments through a more balanced approach taking into account the entire spectrum of deficits observed in this brain disease.

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Contributions: G.D., S.C. and M.S. were responsible for overall study design. G.D., S.C., M.F. and S.B. carried out the stereotaxic surgeries and the post-surgery monitoring of the animals. C.C., G.D. and S.C. carried out the neuroanatomical analysis and characterization of the lesions. S.C. and G.D. performed the experiment for the neurochemical characterization of the lesion and A.B. carried out the HPLC process and analysis. S.C. and G.D. carried out the behavioral experiments and analysis. S.C., M.S. and G.D. wrote the paper with the help of the other authors.

Figure legends

Fig. 1. Bilateral partial lesions of the mVTA or SNc result in distinct, non overlapping, complementary patterns of DA depletion throughout striatal territories. (A and B) Representative photomicrographs of coronal sections stained for TH in striatal (+1.7 to 0.7 mm anterior to bregma) (A) and mesencephalic (-5 to -5.8 mm anterior to bregma) (B) regions according to the stereotaxic atlas of Paxinos and Watson^{36, 37}. Bar = 1 mm. The intensity of the gradient of color (white to green or white to blue) in schematic sections corresponds to the measured DA lesioned area in the different brain structures studied for each lesion performed. mVTA lesion are shown in green and SNc lesion in blue. The highest intensity of green or of blue color (100%) indicates that all animals had lesions in the corresponding area, whereas the lowest color intensity (white, 0%) corresponds to a non lesioned or denervated area. (C and D) Quantification of the loss of TH staining in the different mesencephalic (C) and striatal (D) structures, expressed as percentage of the mean value obtained for sham-operated animals. Two-way ANOVAs revealed significant interactions between the lesion and the brain region considered ($F_s > 9.49$, $P_s < 0.001$). $n = 22-28$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. mVTA, medial ventral tegmental area; NAc, nucleus accumbens; SNc, substantia nigra pars compacta.

Fig. 2. Bilateral partial 6-OHDA lesions of either the mVTA or SNc do not impair locomotion. (A, B) DA lesions did not affect the latency to fall from an accelerating rotarod (A) ($P_s > 0.42$, $n = 22-28$), or horizontal ambulatory activity (B) during a 20 minutes period in an open area (no effect of lesion: $F_s < 0.91$, $P_s > 0.35$ and no lesion x time interaction: $F_s < 0.64$, $P_s > 0.88$, $n = 11-18$). (C) A two-way ANOVA showed a marginal effect of the SNc lesions ($F_{1,144} = 4.04$, $P = 0.05$) and lesion x paw interaction ($F_{3,144} = 2.54$, $P = 0.06$) on the number of adjusting steps. No such effects were found for the mVTA lesions ($F_s < 1.91$, $P_s > 0.18$), $n = 22-28$. (D) SNc lesions had no effect on gait parameters analyzed with an automated gait analysis system (no effect of lesion: $F_s < 2.37$, $P_s > 0.14$ and no lesion x paw interaction: $F_s < 0.92$, $P_s > 0.43$, $n = 12-14$).

Fig. 3. Bilateral partial 6-OHDA lesions of the SNc, but not of the mVTA, decrease consummatory responses, weight gain and social interaction without affecting olfaction. (A, B)

Water (A) and food (B) intake in the homecage were measured over a 24-hour period. (B) Both lesions resulted in a loss of weight after surgery, but mVTA-lesioned animals recovered during the three weeks following surgery, whereas SNc-lesioned animals continued to gain less weight than the controls (Effects of lesion: $F_s > 4.48$, $P_s < 0.05$ and significant lesion x day interaction: $F_s > 5.16$, $P_s < 0.001$). (D) SNc lesions reduced the time spent in social interaction with a congener. (E, F) 6-OHDA lesions did not affect attraction toward an appetitive (coconut, E) or avoidance from an aversive (acetic acid, F) odor $n = 12-18$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Sham-operated vs. Lesioned.

Fig. 4. Bilateral partial 6-OHDA lesions of the SNc, but not of the mVTA, impair motivated behaviors. (A, B) SNc (lesion x session interaction: $F_{6,108} = 2.65$, $P < 0.02$, $n = 19-22$) but not mVTA

(lesion x session interaction: $F_{6,66} = 0.84$, $P = 0.55$, $n = 12-15$) lesions increased the latency to reach a palatable food in a runway paradigm at the asymptotic level (A), with no incidence on CPP for the same reward (B, effect of conditioning: $F_s > 11.61$, $P_s < 0.001$, no effect of lesion: $F_s < 0.50$, $P_s > 0.48$ and no interaction: $F_s < 0.16$, $P_s > 0.69$, $n = 10-17$). (C, D) SNc (* $P < 0.05$) but not mVTA ($P = 0.41$) lesions decreased operant sucrose self-administration (C, representation of the mean of the three last FR1 sessions (first graph) and linear regressions between sucrose deliveries and the loss of TH in the NAc (second graph) and the dorsal striatum (third graph) for mVTA and SNc lesions respectively, $n = 6-9$), while having no significant effect on sucrose preference in a two-bottle choice procedure (D, $P_s > 0.08$, $n = 12-19$). (E) SNc but not mVTA lesions (Effect of lesion: $F_{1,98} = 14.1$, $P < 0.01$ and $F_{1,91} = 0.01$, $P = 0.94$ respectively, $n = 7-8$) reduced self-activation of a cue-light during an operant procedure. Linear regressions between cue-light deliveries and the loss of TH in the NAc and the dorsal striatum for mVTA and SNc lesions respectively. (F) No effects of the 6-OHDA lesions were found on the preference for a novel environment ($P_s > 0.73$), $n = 6-8$. * $P < 0.05$, ** $P < 0.01$, Sham-operated vs. Lesioned.

Fig. 5. Bilateral partial 6-OHDA lesions of the SNc, but not of the mVTA, cause a broad spectrum of affective impairments. (A-C) SNc-lesioned rats displayed increased immobility in the forced-swim test (A, effect of lesion: ($F_{1,47} = 4.71$, $p < 0.05$), no effect of site: ($F_{1,47} < 1$) and a significant interaction between both factors: ($F_{1,47} = 4.15$, $p < 0.05$, $n = 12-19$), and anxiety-like behaviors, as reflected by a reduction in the latency to enter into the black compartment in a light/dark avoidance test (B, effect of lesion: ($F_{1,59} = 4.73$, $p < 0.05$), no effect of site: ($F_{1,59} < 1$) and no significant interaction: ($F_{1,59} = 2.07$, $p = 0.15$, $n = 12-19$) and in the time spent in the open arms of an elevated plus-maze (C, marginal effect of the lesion: ($F_{1,71} = 3.79$, $p = 0.06$), no effect of site: ($F_{1,71} = 0.06$, $p = 0.80$), but a significant interaction between both factors: ($F_{1,71} = 4.37$, $p < 0.05$), $n = 15-22$). No significant linear regressions were found in the forced-swim test (A) and in the elevated plus-maze (C) while a significant negative correlation was found between the latency to enter into the dark chamber in the light/dark avoidance test and loss of TH within the dorsal striatum (B). $**P < 0.01$, Sham-operated vs. Lesioned.

Fig. 6. Effects of L-dopa, ropinirole and citalopram on the behavioral changes induced by the SNc DA lesion. Effects of chronic intraperitoneal administration of L-dopa (12.5 mg/kg), ropinirole (1 mg/kg) or citalopram (10 mg/kg) evaluated in the forced-swim test, (A) the elevated plus-maze and (B) in an operant sucrose self-administration procedure (C). Significant lesion x treatment interactions were found for (A) and (B) ($F_s > 2.94$, $P_s < 0.05$) but not for (C) (significant effect of lesion: $F_{1,63} = 16.53$, $P < 0.001$, but no interaction: $F_{3,63} = 0.78$, $P = 0.51$). $n = 6-11$. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, Sham-operated vs. Lesioned.

Online Methods

Animals

Experiments were performed on male Sprague Dawley rats (Janvier, Le Genest-Saint-Isle, France) weighing 180 g (6 weeks old) at the time of surgery. Animals were housed four per cage until the second week after surgery and then transferred to individual cages until the end of the study, under standard laboratory conditions (12 h light/dark cycle, with lights on at 7:00 am) with food and water available *ad libitum*, unless otherwise stated. Protocols used complied with the European Community Council Directive of 24 November 1986 (86/609/EEC) for the care of laboratory animals, French Ministry of Agriculture regulations (authorization no. 38-R1001) and French guidelines for the use of living animals in scientific investigations. They were approved by the Grenoble-Institut des Neurosciences ethics committee, under agreement number 004.

Bilateral 6-OHDA lesions

All animals were anesthetized with a mixture of xylazine (15 mg/kg i.p.) and ketamine (100 mg/kg, i.p.) and treated with desipramine hydrochloride (25 mg/kg s.c.; Sigma, St Quentin-Fallavier, France) to protect noradrenergic neurons³⁸, 30 min before 6-OHDA injection. Rats were secured in a Kopf stereotaxic apparatus (Phymep, Paris, France) and 6 µg of 6-OHDA dissolved in 2.3 µl of sterile 0.9% NaCl with 0.2% ascorbic acid (Sigma, St Quentin-Fallavier, France) were injected bilaterally, at a flow rate of 0.5 µl/min. The solution was delivered to the medial plane, to target the medial ventral tegmental area (mVTA group), or into the medial part of the substantia nigra pars compacta (SNc group). The stereotaxic coordinates of the injection site relative to bregma were as follows, according to the stereotaxic atlas of Paxinos and Watson (1998)³⁶: (1) mVTA lesion: anteroposterior (AP), -5.6 mm; lateral (L), +1.0 mm, with a 10° angle toward the midline, and dorsoventral (DV), -8.1 mm; (2) SNc lesion: AP, -5.4 mm; L, ±1.8 mm and DV, -8.1 mm, with the incisor bar at +3.2 mm below the interaural plane. After each injection, the cannula was left in position for 5 min to allow the injected solution to be absorbed and to minimize the spread of the toxin along the needle tract. An identical procedure was used for sham-operated controls, but with 2.3 µl of vehicle (0.9% NaCl, 0.02% ascorbic

acid). After recovery from anesthesia, animals returned to the facility for three weeks, to allow the 6-OHDA lesion to develop and stabilize³⁸, before the beginning of the behavioral experiments.

Transient starvation states occurred two to three days after surgery in a subset of SNc-lesioned animals (around 20%). These animals received supplementation with a high-caloric liquid diet and palatable food for 1 to 2 weeks. Animals that did not recover (< 5%) were discarded from the experimental procedure. This phenotype was never observed in the Sham and mVTA lesions groups.

Histological analysis

Immunohistochemistry. Immunohistochemical analysis was carried out as previously described³⁹. Briefly, rats were killed under chloral hydrate anesthesia at the end of the behavioral experiments, perfused intracardially with paraformaldehyde and brains removed. Free-floating 30 µm-thick coronal sections from the mesencephalon and the striatum were incubated with an anti-TH antibody (mouse monoclonal MAB5280, Chemicon, Temecula, USA; 1:2500), and then with a biotinylated goat anti-mouse IgG antibody (BA-9200, Vector Laboratories, Burlingame, CA, USA; 1:500). Immunoreactivity was visualized with avidin-peroxidase conjugate (Vectastain ABC Elite, Vector Laboratories, Burlingame, CA, USA).

Quantification of the extent of the mesencephalic DA lesion and of striatal DA denervation.

TH-immunolabelling detection of DA neurons and terminals were evaluated under a light microscope (Nikon, Eclipse 80i) coupled to the ICS FrameWork computerized image analysis system (TRIBVN, 2.9.2 version, Châtillon, France).

For quantification, six selected TH-labeled coronal sections for each experimental animal, corresponding to three antero-posterior levels of the striatum (+0.7 to 1.7 mm anterior to bregma) and of the mesencephalon (-5 to -5.8 mm anterior to bregma), were digitized with a camera (Pike F-421C, ALLIED Vision Technologies, Stadtroda, Germany). For each section, six subregions within the striatum and three subregions within the mesencephalon were chosen, taking into account the topography of DA innervation^{2, 40}, as indicated in Fig. S1A and B.

For all quantitative measurements, masks from these different striatal and mesencephalic subregions were drawn with the computer analysis system to ensure that appropriate comparisons were made between homologous anatomical regions. Optical densities (OD) were measured for each striatal and mesencephalic subregion, and the mean OD was calculated with ICS FrameWork software (TRIBVN, 2.9.2 version, Châtillon, France). OD values were measured for the denervated and non denervated territories of the lesioned animals for each section analyzed and were compared with those for the homologous regions in sham-operated animals. The OD value obtained for an unlabeled area (the corpus callosum) was used as the background and was subtracted from each of the OD values measured.

A simplified and classic subdivision of mesencephalic and striatal areas is shown in Fig. 1, for conciseness and to take the nigrostriatal and mesolimbic projections into account.

Brain tissue dopamine determination

Three weeks after surgery, dorsal striatum and NAc were dissected out and processed as previously described⁴¹, for the determination of DA concentration with a liquid chromatography system (Shimadzu, France) coupled to an electrochemical detector (Decade, Antec Leiden, the Netherlands) and a C18 reverse-phase microcolumn (Aquasil, RP-18, 150×1 mm, 3 µm particle size, ThermoHypersil, maintained at 30°C). The mobile phase (50 mM NaH₂PO₄, 0.1 mM EDTA-Na², 1.7 mM sodium octyl sulfate, 4.5 mM KCl and 5% acetonitrile (vol/vol), adjusted to pH 3.10) was run at a flow rate of 0.06 ml/min. DA contents were determined by comparing DA peaks with external standards and were expressed as the amount of DA, in ng per mg of brain tissue.

Behavioral procedures

All rats were subjected to a sequence of behavioral tests, as summarized in Fig. S2. Different groups of animals were exposed to different sequences, as some combinations of tests, such as operant sucrose self-administration and cue-light self-administration or conditioned place preference (CPP) and novelty-preference, could not be performed on the same animal. However, all animals were

tested for potential locomotor deficits on the rotarod and in the stepping test. When animals were subjected to a long sequence of behavioral tests or pharmacological treatment, two subgroups of animals were constituted, and the order of the tests was reversed in the second subgroup (see group A and B in Fig. S3A and B), to ensure that the effects of the lesion or of a pharmacological treatment did not change over the course of the study. No effect of order was found, in any of the conditions tested (data not shown). In each experiment, all conditions (lesions and/or pharmacological treatments) were counterbalanced among the different test chambers according to a Latin-square design. Each apparatus was cleaned with 10% ethanol and 2% H₂O₂, and dried with a paper towel after each trial or session.

Accelerating rotarod. Animals were first trained to remain on a rotarod (Harvard Apparatus, Holliston, MA, USA) turning at 4 rpm for more than 30 s. The rotation speed of the rod was then gradually increased, at a rate of 1 rpm every 8 s. Latency to fall from the rod was recorded three times for each rat.

Stepping test. Animals were moved sideways along a smooth-surfaced table number of forelimb adjusting steps measured, as described by Olsson *et al.*, 1995⁴². The test was carried out three times for each paw, by two experimenters blind to the experimental conditions.

Gait analysis. Gait was analyzed with an automated gait analysis system (GaitLab, Viewpoint S.A., Champagne au Mont d'Or, France). Rats were imaged from below, with a high-speed camera (~150 frames per second), while they ran on a narrow glass corridor (7 × 90 cm), to identify paw step positions and moving speed. Different metrics were calculated, including speed, stride length, stance time, swing time and number of strides per second. After a period of training of one week, gait and ambulatory behaviors were recorded three times for each rat, on a final test day.

Locomotor activity. Rats were placed in a dimly lit white Perspex™ open arena (50×50×40 cm) and horizontal distances traveled were recorded with a video-tracking system (Viewpoint S.A., Champagne au Mont d'Or, France), over a 20-minute period.

Olfactory tests. Tests were carried out in a dimly lit white open arena, with a video-tracking system. Olfactory avoidance and preference behaviors were evaluated by comparing the time the rats spent near two filter papers soaked in 40% acetic acid or in 50% coconut milk with the time spent near two filter papers soaked in distilled water. Acetic acid and coconut milk were used as they have been shown to be potent olfactory aversive⁴³ and attractive⁴⁴ cues, respectively.

Runway task for food. Food-restricted rats (90% of their free-feeding weight) were trained to run from a start box (20x15x40 cm) to the end of a Perspex™ alley (100x15x40 cm) to obtain a palatable food (salted, cheese-flavor cookies, Belin, France) presented in a plastic bowl. Ambulatory pattern was recorded, together with the latency to reach the food at the end of the runway and to start to eat it, with a video-tracking system. Animals were allowed to eat for less than 30 s, to prevent early satiation¹⁶. A 120 s cut off was used when animals did not complete the task. This procedure was repeated three times per day over a period of seven days. Performances were represented as a completion score¹⁶: $(120 - \text{latency to reach food})/120 \times 100$.

Conditioned place preference for food. CPP chambers consisted of two compartments (40x33x35 cm) differing in wall colors and floor texture, separated by a small (10 cm length) compartment⁴⁵. A video-tracking system was used to measure the time spent in each compartment. During a preconditioning session, food-restricted rats (90% of their free-feeding weight) were placed in the CPP chamber and allowed to freely explore the three compartments for 15 minutes. Conditioning took place over eight consecutive days. During these sessions, animals were confined alternatively to one compartment with palatable food (Belin, France) for the paired condition, or without food for the unpaired condition, and to the other without food for both the paired and unpaired conditions. For

testing, rats were allowed to explore the entire chamber for 15 minutes, as during the preconditioning session. Preference scores were expressed as the difference between the time spent in the food-paired compartment during the CPP test and the preconditioning test.

Evaluation of sucrose preference. Rats were given 24-h concurrent access in their home cage to two graduated 250 ml plastic bottles (Techniplast, France), for three days. One of these bottles contained tap water, whereas the other contained 2% sucrose (Sigma, St Quentin-Fallavier, France) in tap water. Rats and bottles were weighed daily, with the position of the bottles (left or right) alternated, to control for side preference. The first day was used as an acclimation period. The volumes of sucrose solution and water consumed on the second and third days were averaged to determine sucrose, water and total fluid intake (ml/kg), and preference for sucrose over water (sucrose intake/total intake, expressed as a percentage).

Evaluation of saccharin preference. Rats were given 24-h concurrent access to a bottle containing tap water and another containing 0.002% saccharin (Sigma, St Quentin-Fallavier, France) in tap water, using a procedure similar to that for sucrose. Rats were then given access to two bottles, containing 0.002% and 0.02% saccharin, respectively, for the next three days.

Operant sucrose self-administration. Rats were first habituated to voluntary consume 2% sucrose solution in a two-bottle choice procedure, as described above. They were then trained to self-administer a 2% sucrose solution in operant chambers (Med Associates, St. Albans, VT, USA) as previously described⁴⁶, under a fixed ratio 1 reinforcement schedule (FR1), with an active, reinforced, lever, for which presses resulted in the delivery of 0.2 ml of the sucrose solution, and an inactive, non reinforced, lever. Once performances had stabilized (less than 20% performance variation over three consecutive sessions), rats were subjected to a progressive ratio schedule session, in which the number of active lever presses required for a reward increased exponentially after each reward, according to Roberts' equation⁴⁷. The session ended when the rat failed to complete a response

requirement (i.e., a ratio) within one hour. The breakpoint was defined as the final ratio completed by the animal⁴⁷.

Cue-light self-administration. A naive set of rats (i.e., that had not been subjected to the operant sucrose self-administration procedure) was tested daily for 1 h, during 8 days, for lever responses to the contingent presentation of a 6 s cue-light (FR1), located 4 cm above the active lever, in the self-administration chambers described above. The sides on which the inactive and active levers were located (right or left), were distributed evenly between the different conditions.

Novelty preference. This procedure was adapted from a method described elsewhere⁴⁸. We used the same place preference chambers as described above for the CPP for food, with the same video-tracking system set-up. Rats were pseudorandomly exposed for 20 min to one compartment ('familiar'). At the end of this habituation phase, animals were allowed to explore the whole chamber (familiar and new compartments) for 15 min. A novelty preference index was calculated as follows: $\text{time spent in the new compartment} / (\text{time spent in the new compartment} + \text{time spent in the familiar compartment}) \times 100$.

Social interaction. Rats were placed in a dimly lit white Perspex™ arena (50x50x45 cm), for 10 min, for acclimatization to their surroundings. An unfamiliar male congener was then introduced into the arena and social interaction was video-recorded for 10 min. The total time that the test rats spent engaging in social interaction behaviors (listed and defined in⁴⁹) was scored by two observers blind to the experimental conditions.

Elevated plus-maze. The elevated plus-maze (Viewpoint S.A., Champagne au Mont d'Or, France) consists of two opposing open arms and two opposing arms enclosed by 40 cm high walls, and was placed in a dimly lit room. Each arm was 50 cm long and 10 cm wide and made of black Perspex™, suspended 55 cm above the floor. The rats were placed in the center of the maze and their behavior

was recorded for 5 minutes with a video-tracking system. Number of entries into and total time spent in the open and closed arms were quantified by the video-tracking system.

Light/dark avoidance test. The apparatus was made of Plexiglas and consisted of a light and a dark chamber (38x33x35 cm each), separated by an opaque Plexiglas wall with a 7 x 7 cm aperture, to allow the animals to move freely between the chambers. The light chamber was made of white walls, opened at the top, and was lit with a white incandescent light (100 watts) located 70 cm above the floor of the chamber. By contrast, the dark chamber was made of black walls, closed at the top, and was not lit. The animals were placed in the center of the light chamber, facing away from the opening toward the dark chamber, and were video-recorded for 5 min. Latency to the first entry into the dark chamber and the total amount of time spent in the light chamber were determined by two observers blind to the experimental conditions.

Forced swim test. Rats were placed in a cylinder of 40 cm high and 20 cm in diameter, filled with water ($24\pm 1^{\circ}\text{C}$) to a depth of 30 cm, for 15 min. Twenty four hours later, they were placed in the same cylinder for 5 min. Animal activity was detected and recorded with a video-tracking system.

Pharmacological procedures. Three weeks after 6-OHDA infusion, we initiated a sequence of behavioral tests on mSNc-lesioned rats, as described in Fig. S2B. Intraperitoneal administration of 12.5 mg/kg of L-dopa (together with 15 mg/kg of benserazide), 1 mg/kg of ropinirole, 10 mg/kg of citalopram, or vehicle (0.9% NaCl), at a volume of 1 ml/kg, began two days before the start of the behavioral tests sequence. Injections were carried out 30 min before the beginning of each behavioral session.

Data and statistical analysis

Data were analyzed by *t*-tests or two-way ANOVAs, depending on the experimental design. When indicated, *post-hoc* analyses were carried out with the Bonferroni's correction procedure or the method of contrasts. Dimensional analyses were performed by parametric simple linear regressions.

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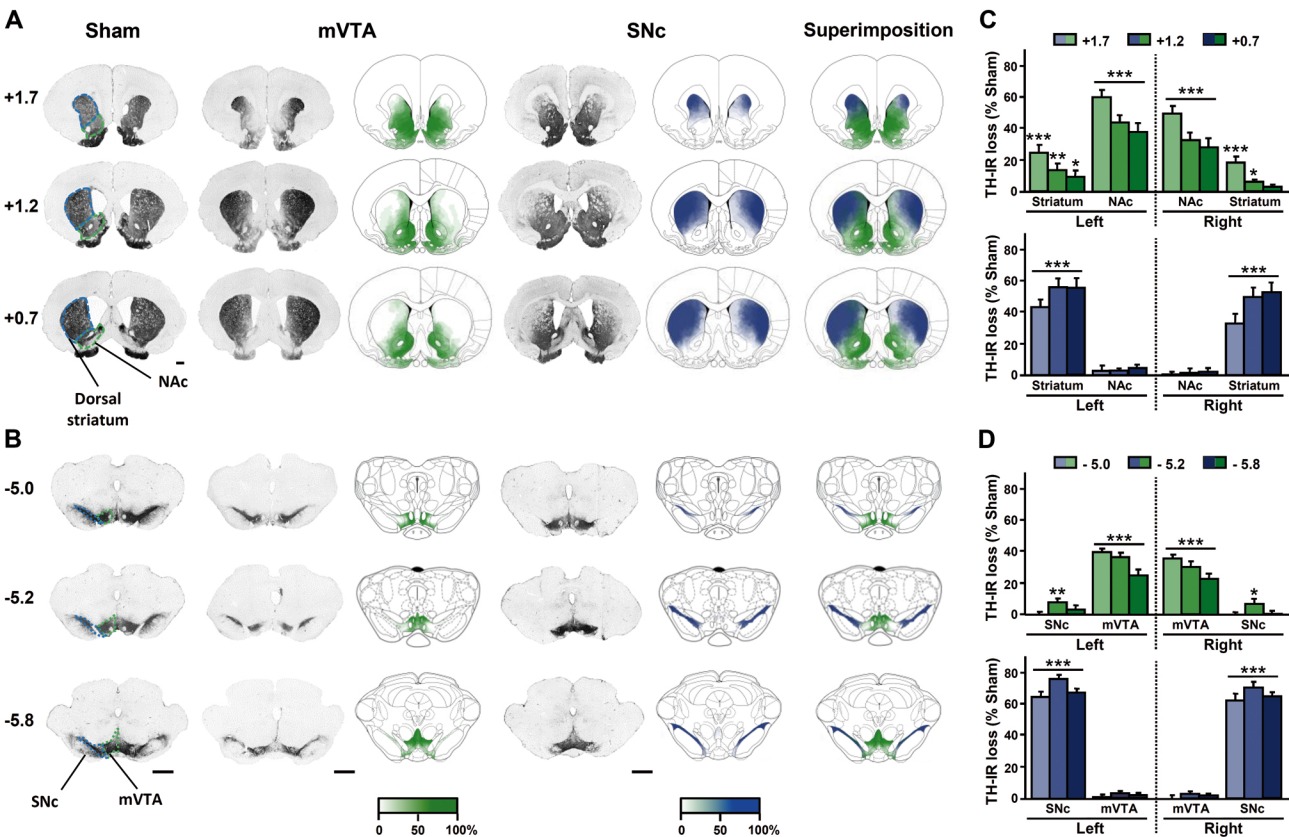
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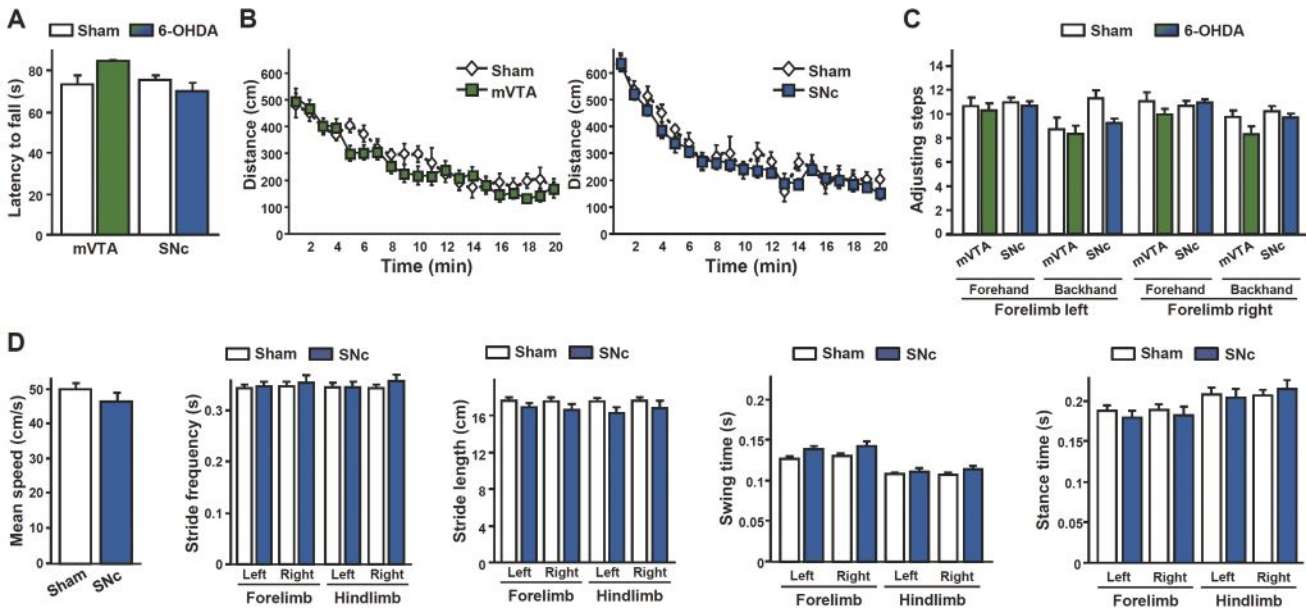
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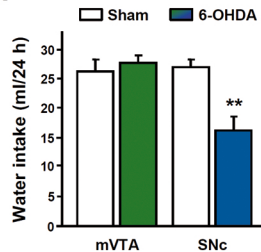
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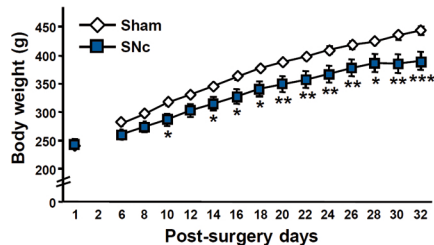
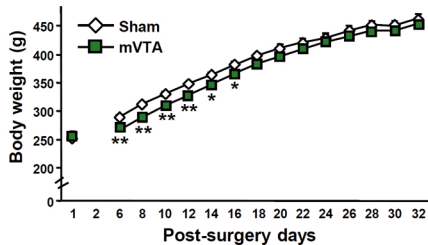
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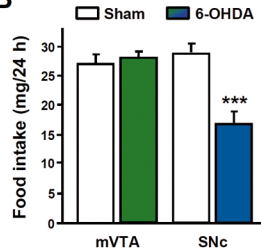
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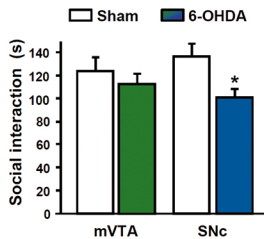
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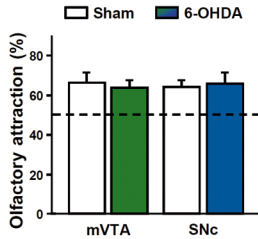
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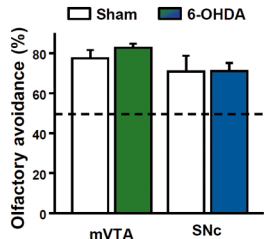
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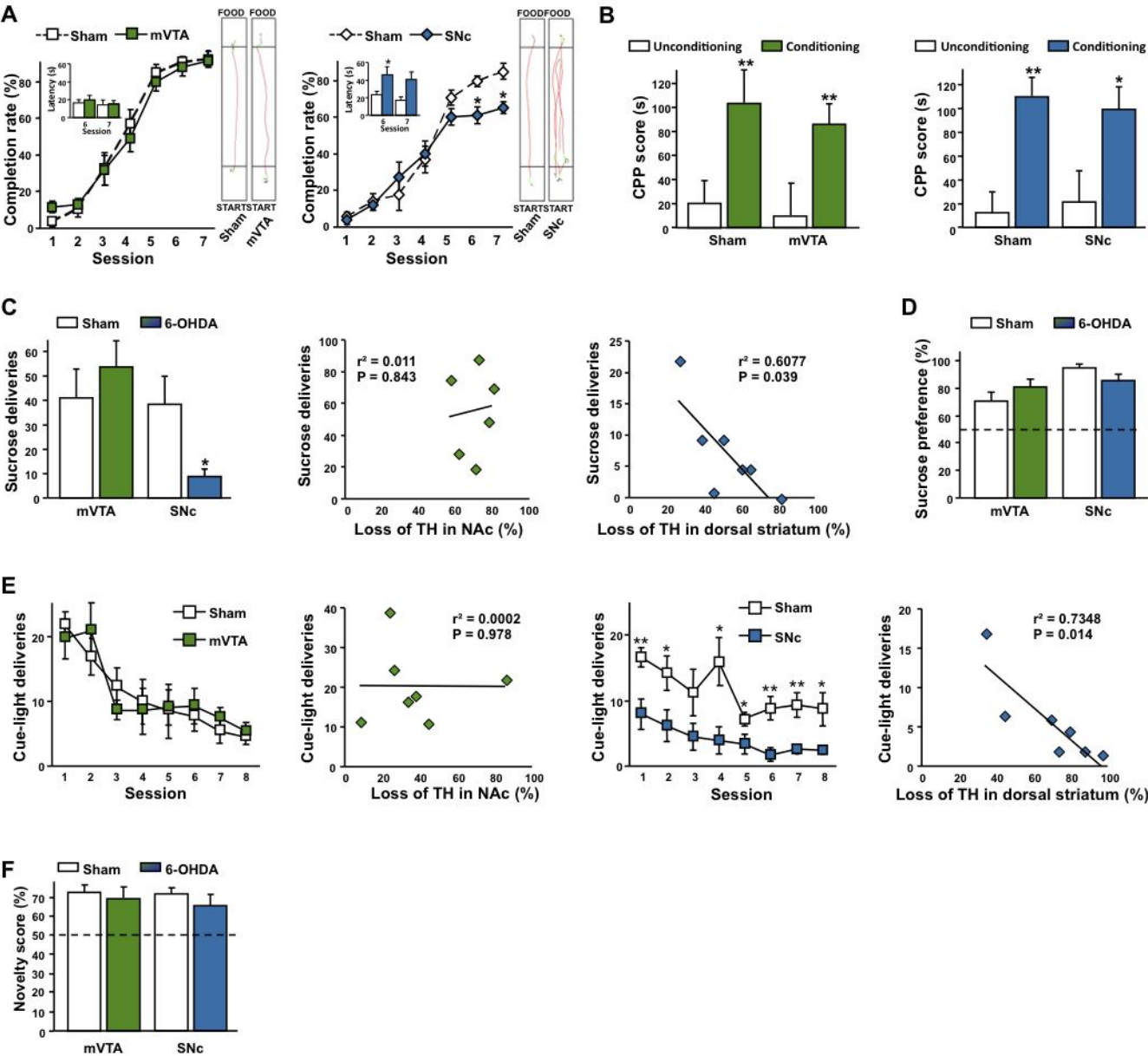


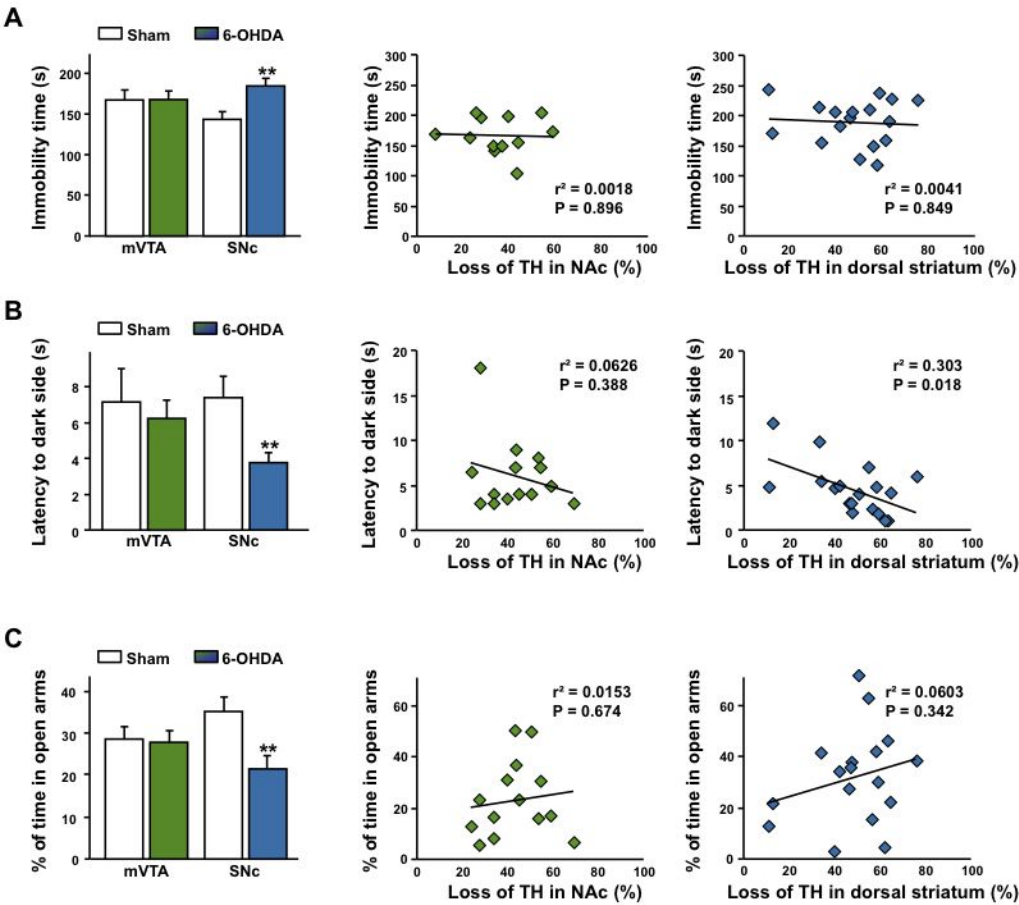
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